

Colony Formation via Sexual and Asexual Reproduction in *Salmacina dysteri* (Huxley) (Polychaeta, Serpulidae)

EIJIROH NISHI¹ and MORITAKA NISHIHIRA²

¹Amakusa Marine Biological Laboratory, Kyushu University, Amakusa, Kumamoto 863-25 and ²Biological Institute, Faculty of Science, Tohoku University, Aoba-ku, Sendai 980, Japan

ABSTRACT—Reproduction and colony formation in the tubicolous serpulid polychaete *Salmacina dysteri* (Huxley) were investigated on the Okinawan coral reef. Numerous individuals of this species aggregate and construct an arborescent pseudo-colony which is formed by physiologically unconnected individuals. This species reproduces both sexually and asexually. The proportion of the two modes of reproduction varied according to the position of individuals in the colony. Growing tips of colony branches included more asexually reproducing worms, while basal or central parts of a colony contained more sexually mature ones. The predominant mode of reproduction in a colony changed with colony size. In colonies with < about 500 worms, asexual reproduction was more frequent than sexual reproduction, but in colonies with >2,000 worms, sexual reproduction predominated.

INTRODUCTION

Annelids show various modes of reproduction, such as sexual and asexual reproduction, dioecism and hermaphroditism, brooding and broadcast spawning, viviparity, external and internal fertilization, etc. [18]. Among the polychaetes, asexual reproduction is least mode of reproduction. Detailed reproductive processes have been reported in many polychaete families, and the colonial status was achieved through asexual budding in cirratulids [7], spionids [21], sabellids and serpulids [8]. Asexual cloning is seen in *Dodecaceria concharum* and *D. fewkesi* [1, 7], and *D. fewkesi* builds separate male and female colonies through asexual reproduction [1]. Gibson [7] noted that, the colonies of all asexually reproducing species of *Dodecaceria* can theoretically be produced from a single ovum. The relationship between asexual cloning and colony forming process has not been previously studied in polychaetous annelids.

Individuals of *Salmacina dysteri* form calcareous, arborescent aggregations, which lack physiological connections between individual worms [6, 15]. We use the term "pseudo-colony" for such an aggregation according to Knight-Jones & Moyses [10]. *Salmacina dysteri* can perform various reproductive modes; worms may be male, female, or hermaphroditic, and reproduce asexually budding [14, 15]. The interaction among these modes has not been known up to now in pseudo-colonial polychaetes, though interaction of sexual and asexual reproduction has been detected in eu-colonial organisms, which have physiological connections, especially in relation to the age or size of the colony [2, 11].

In the present study, we analyze the interaction of sexual and asexual reproduction according to size or age of the pseudo-colony of *S. dysteri*.

SAMPLING SITES AND MATERIALS

Field studies were conducted on Sesoko Island (26° 38'N, 127° 52'E), Okinawa, South-western Japan (Fig. 1). Two study sites were selected; 1) a mass of a concrete block artificial reef set off Sesoko Island, and 2) the vertical wall of the pier in Hamasaki Port. The former was at a depth of about 10 m, the latter 2 m to 4 m. The

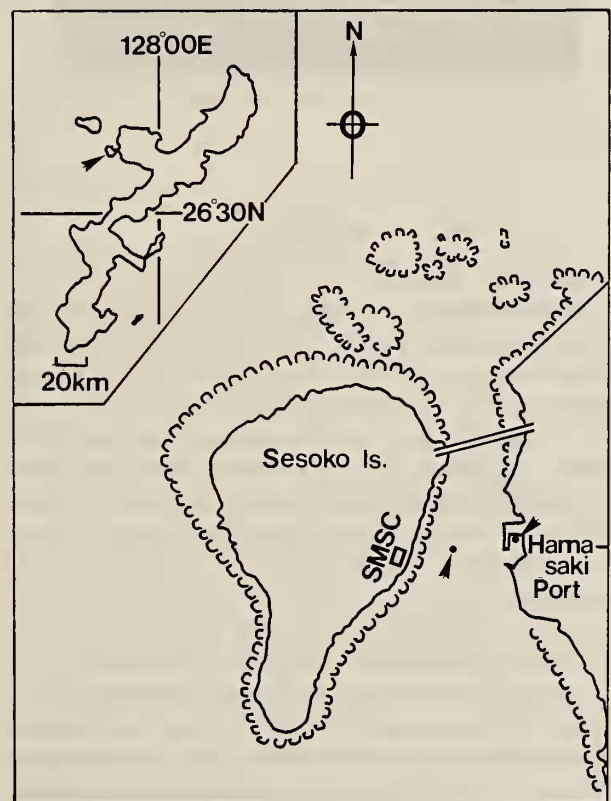


FIG. 1. Map of the study area. Arrow in the inserted map shows Sesoko Island, two dots pointed with arrows show the sampling points. SMSC, Sesoko Marine Science Center.

bottoms of both sites were covered by sand or mud substrata. The sea water temperature of these sites ranged from 18 to 32°C during the year [12]. The pseudo-colonies of *Salmacina dysteri* were found attaching to hard substrata such as dead coral skeletons, concrete blocks, bivalve shells, etc. at a depth of 5 m to 10 m.

Pseudo-colonies of *S. dysteri* were arborescent, and looked like a dead eu-colony of a finely branching coral *Seriatopora hystrix* (Fig. 2; similar shaped pseudo-colonies were found at the port of Iwaya, Awajishima, Central Japan [22]). Pseudo-colonies 5 to 10 cm in diameter (which include 500 to 2000 worms per colony) were common. The size of a mature individual is 2 to 3 mm in length including a branchial crown, and 0.2 mm in width. The branchial crown consists of 4 pairs of white filaments, an operculum is absent [9]. The thorax and abdomen are red, each having 6–7 and 8–25 segments respectively.



Fig. 2. Arborescent colony of *Salmacina dysteri*, about 20 cm in diameter.

METHODS

Identification of species

Salmacina dysteri and sympatric related species can only be separated based on their collar setal morphology or teeth distributional pattern [13]. Scanning electron microscopy (SEM) was used for the identification of *S. dysteri*. For SEM, worms were fixed in 2.5% glutaraldehyde buffered in seawater, rinsed, dehydrated, dried with a critical-point dryer using liquid CO₂, and coated with gold-palladium. Usually, 5 to 20 worms were selected at random from a pseudo-colony, then viewed with the SEM and checked for their collar setal morphology.

Inter-colonial variation of the proportion of juveniles

The number of juveniles with a small body was recorded separately from the number of adults. Pseudo-colonies were collected between April and June 1989. Juveniles were separated by their body size into two categories. Juveniles which were sexually produced had 3 pairs of branchial filaments and a thin semi-transparent tube [16]. Their thoracic and abdominal segments were short and had fewer

segments (3–8) than adults (10–25). By contrast, juveniles which had originated from buds by asexual reproduction had 8 branchial filaments [13] and wide abdominal segments which were comparable to those of adults. These asexually produced juveniles had 4–5 thoracic and 7–10 abdominal segments. After 1 month following settlement of larvae or 2 weeks from the release of a bud from the stock, these juveniles could not be identified from each other or from adult worms.

Inter- and intra-colonial variation of reproductive modes

In order to know the inter- and intra-pseudo-colonial variations of reproductive modes, the numbers of sexually mature and asexually reproducing worms were recorded for some pseudo-colonies. Materials were collected between April and June 1989 to minimize seasonal effects. Individuals in the male phase have white segments, whereas those in the female phase have brown ovoid eggs in the coelom or the tube. Asexually reproducing worms have a bud in various developmental stages in their abdomen. If the worm with adult size and morphology lacked a bud on its abdomen or eggs and embryos in the abdomen or tube, it was treated as an immature worm or juvenile. In this paper, sexually mature worms represent, male, female, and hermaphroditic worms. Asexually reproducing female or male worms rarely appeared (unpublished data). However, the samples analyzed in this study did not contain a worm reproducing sexually and asexually simultaneously.

Experimental halving of pseudo-colony

To study the effect of pseudo-colony size on the reproductive mode, six pseudo-colonies with various sizes (ranging from 8–11 cm, averaging 9 cm in diameter) were selected, and about half of each colony was sampled to check the proportion of reproductive worms. The remaining half (about 4 cm in diameter) was left as it was on the substrate. Six weeks later, these latter halves were collected to check the proportion of reproductive worms. This field experiment was conducted between July and August, 1989. About 50–80% of worms (100 to 200 worms) in each pseudo-colony were examined under a dissecting microscope. The worms were collected from all positions of a colony to minimize intra-pseudo-colonial variation of reproductive modes.

Effect of pseudo-colony age on reproductive modes

In order to know the effect of age on reproductive modes, the relationship between progressed time (month) from initial observation and reproductive ability was tentatively calculated from field experiments conducted between April 1989 and July 1990. Seventeen small pseudo-colonies about 3 to 5 cm in diameter (with an estimated age 2 to 5 months from settlement) were selected, then only a part of a branch (10 to 50 worms) was collected and checked for reproductive ability. All the colonies remained in the field. After some (1 to 6) months, 2, 3 or 4 colonies were re-collected and checked for their reproductive activity. This

experiment was initiated in spring or summer months. When we check their reproductive ability, because intra-colonial variation of reproductive modes may appear [14], worms were collected from various positions (peripheral, middle and central positions) within a pseudo-colony.

Induction of asexual budding in solitarily reared worms

A total of 70 sexually mature worms with calcareous tubes were reared solitarily for 1 month in glass dishes (8 cm in diameter, 10 ml in capacity) in a natural light regime. The water temperature was 22–28°C. Diatoms were added as food each week. Ten fragmented branches (containing 10 to 30 worms) with calcareous tubes were taken from a central part of a large colony about 10 cm in diameter. They were then also reared in separate dish after identifying sex of each worm if possible.

Aggregation during larval settlement

In order to know whether larvae aggregate during the settlement event, trochophore larvae at the 3 setiger stage were gathered from 5 pseudo-colonies by breaking the tubes of brooding females, and released in 4 sets of petri-dishes (9 cm in diameter, 120 ml in capacity). After 1 week, almost all of the larvae had settled, gregariously or solitarily. If tubes of settled worms touched other tubes, it was categorized as aggregation.

RESULTS

Intra-colonial variation of two reproductive modes

Within a single pseudo-colony, particularly larger ones, peripheral parts had a higher proportion of asexually reproducing worms than basal and lower middle parts (Benferri's *t*-test, $P < 0.05$, Fig. 3). The proportion of sexually mature worms was higher in the basal and lower middle parts (Benferri's *t*-test, $P < 0.05$). Between the lower and upper middle parts, the difference in the proportions of sexually mature and asexually reproducing worms were not significant (Benferri's *t*-test, $P > 0.05$).

Inter-colonial variation of the proportion of juveniles

It was possible to discriminate between juveniles produced sexually and asexually. In a single pseudo-colony, there were both juveniles produced by sexual and asexual reproduction in various proportions (Fig. 4). The proportion of sexually produced juveniles was higher in larger colonies than in smaller ones. The positive correlation between the proportion of sexually produced juveniles and colony size was significant ($r = 0.678$, $P < 0.01$).

Inter-colonial variation of two reproductive modes

The pseudo-colonies of *S. dysteri* consisted of many worms in various stages of their life cycles. Newly formed smaller pseudo-colonies (<200 worms) usually contained mostly sexually immature and asexually reproducing worms, but larger ones (>2000 worms) contained abundant her-

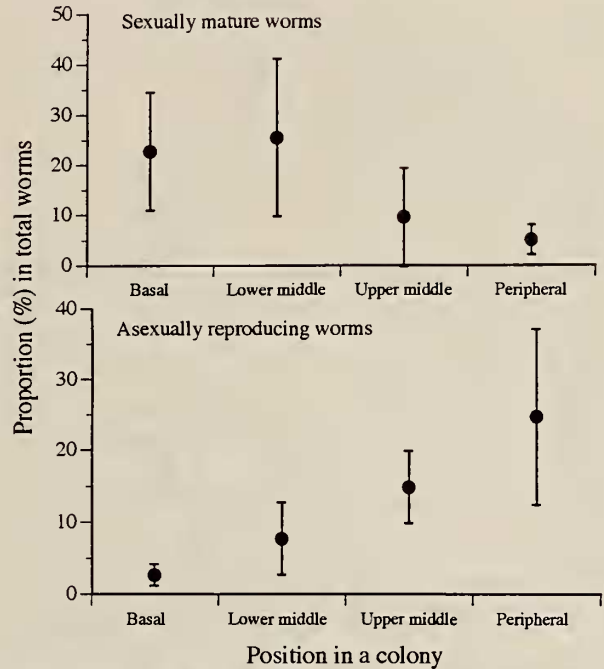


Fig. 3. Proportion of sexually mature and asexually reproducing worms in different parts of 3 colonies of *Salmacina dysteri*. Three colonies were examined between April and June (colonies 5, 8, and 10 cm in diameter), 1989. Ten to 20 worms were examined from 4 parts of each colony, vertical line shows the S.D.

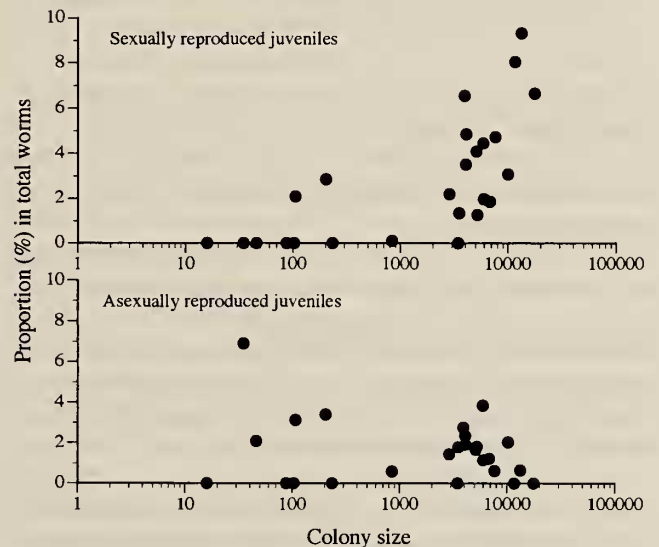


Fig. 4. Relationship between colony size (in terms of number of worms) and proportions of sexually (top graph) and asexually (lower graph) reproduced juveniles in *Salmacina dysteri*.

maphroditic worms (Fig. 5). The proportion of hermaphroditic worms seems to be correlated with pseudo-colony size (in terms of total number of worms) ($r = 0.802$, $P < 0.01$). However, the correlation between the number of males and pseudo-colony size was not significant ($r = 0.385$, $P > 0.05$). The proportion of asexually reproducing worms

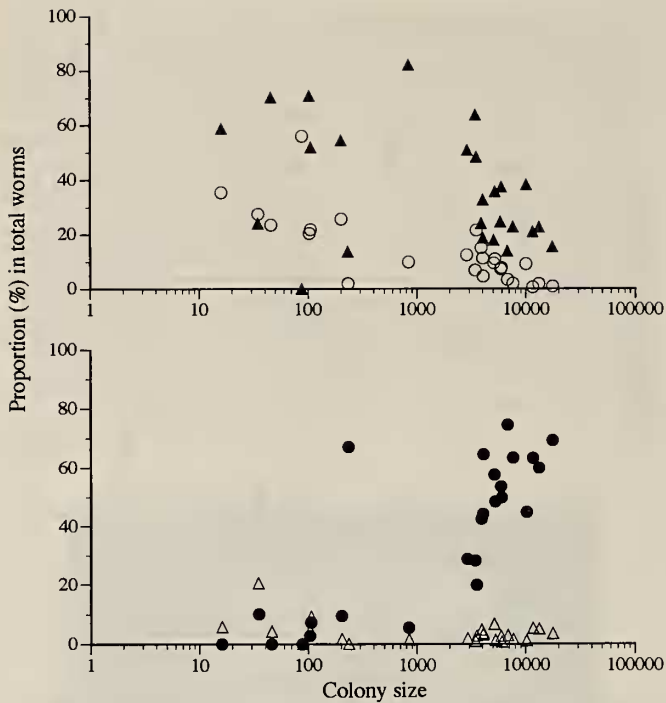


FIG. 5. Relationship between colony size (in terms of the number of worms) and abundance of reproducing worms which have a normal adult shape (with 8 branchial filaments and >10 abdominal segments) in *Salmacina dysteri*. All colonies were collected between April and June, 1989. Whole worms were examined except for the largest 5 colonies where only 60 to 70% were checked. ○, asexually reproducing worms (upper graph); ▲, immature worms (upper graph); △, males (lower graph); ●, hermaphroditic worms (lower graph).

was negatively correlated with the size of the pseudo-colony ($r = -0.759$, $P < 0.01$).

Relationship between estimated pseudo-colony age and reproductive modes

The relationship between pseudo-colony age (expressed by month from initial observation) and the proportion of the mode of reproduction is depicted in Fig. 6. A pseudo-colony survived a maximum of 6 months from the initiation of observation. The growth rate of the pseudo-colony was roughly estimated. The growth of the pseudo-colony was about 1–2 cm in diameter per month.

Since the growth of pseudo-colonies was not studied from the initiation of the pseudo-colony formation (i.e., settlement of larvae), the age of pseudo-colonies was estimated from the preliminary growth experiment (unpublished data); the age of pseudo-colonies with a diameter of 1–2 cm (2 to 10 worms in a pseudo-colony) seemed to be 1 month from the settlement, that with 3 cm in diameter (about 20 to 30 worms) 2 months, and that with a 4 cm diameter (about 40 to 60 worms) 3 months. The pseudo-colonies with an estimated age of about 3 to 5 months (1 to 3 months in Fig. 6) contained few sexually reproduced worms, but contained many asexually reproducing worms (20–30% of the total number). In pseudo-colonies more than 6 months (4 to 6

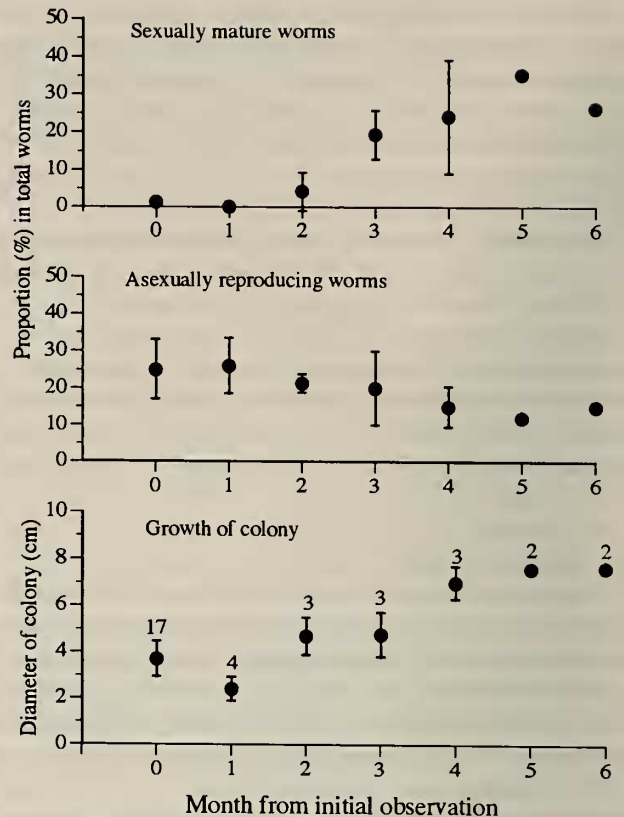


FIG. 6. Relationship between months from initial observation and proportion of sexually (male plus hermaphroditic worms) (upper graph) and asexually (middle graph) reproducing worms in *Salmacina dysteri*. Observations were conducted between April, 1989 and July, 1990, and initiated from small size, 3 to 5 cm in diameter (estimated age of which being 2 to 5 months after the settlement). After some months, worms representing about 20 to 50% of the total worms in a collected colony were examined. Worms were collected from basal, middle, and peripheral parts of the colony to mitigate intra-colonial reproductive variation. The number on the bar in the lower graph shows the number of colonies analyzed. Lower graph; change in colony size (mean \pm S.D.) during observation.

months in Fig. 6), both sexually mature and asexually reproducing worms were found, but the former were more abundant than the latter. The proportion of sexually mature worms (male and hermaphroditic worms) increased with time from initial observation ($r = 0.757$, $P < 0.01$). The proportion of asexually reproducing worms showed a negative correlation with time from initial observation ($r = -0.589$, $P < 0.01$).

Experimental halving of pseudo-colony size

The broken pseudo-colonies still showed a significant sexual reproductive capability 2 weeks after the artificial break, but within 1 to 2 months, they increased the proportion of asexual reproduction to a degree comparable to that of pseudo-colonies of the same size class (Table 1). The decrease in the proportion of sexually mature worms appeared in all pseudo-colonies examined. On the contrary, an increase in proportion of asexually mature worms was detected

TABLE 1. Sexually mature and asexually reproducing worms before and after the experimental halving in 6 pseudo-colonies of *Salmacin adysteri*

Colony	Diameter (cm) of colony	Proportion (%) of sexually mature worms		Proportion (%) of asexually reproducing worms	
		before	after	before	after
1	12	65	37	28	61
2	11	64	47	24	42
3	10.5	24	18	15	34
4	9	54	10.5	22	24
5	8.5	30	21	20	18
6	7.5	17	8	18	21
Average		42.3	23.5	21.2	33.3
S.D.		21.2	15.4	4.6	6.6

TABLE 2. Settlement of 3-setigerous larvae of *Salmacina dysteri* in a petri-dish, known number of larvae were released and settlers were counted after 1 week

Replicates	No. of larvae		Number of aggregations	No. of worms in an aggergation		No. (%) of worms solitarily settled
	released	settled		mean \pm S.D.	range	
1	50	45	7	3.6 \pm 1.59	2-6	21(46.6)
2	50	42	5	4.8 \pm 2.40	2-8	18(42.8)
3	40	25	3	5.3 \pm 2.86	2-9	9(36.0)
4	90	71	8	5.3 \pm 3.56	2-11	29(40.8)

in all colonies examined except for one pseudo-colony. The average changes were not significant in both sexually mature and asexually reproducing worms (Benferroni's *t*-test, $P > 0.05$).

Induction of asexual budding in solitarily reared worms

In the laboratory culture of solitary worms, sexually mature worms were observed to absorb gonads and produce buds within 2 to 3 weeks after the start of the culture (25 among 70 worms). In the culture of fragmented branches, almost all the males and hermaphroditic worms ceased sexual reproduction and started to produce buds for asexual reproduction after 3 to 4 weeks from the start of the laboratory

culture, or produced large amounts of calcareous tubes after the experimental fragmentation.

Aggregation during larval settlement

The larvae settled solitarily or in aggregation with a varying number of individuals (Fig. 9 & Table 2). The difference between numbers of settled worms solitarily and those in aggregation was not significant (Chi-square and *G*-test, $P > 0.05$). Aggregations were formed mostly on the vertical wall of a petri-dish. Only 5 aggregations were formed on the bottom.

DISCUSSION

Intra-colonial variation of the two reproductive modes

The proportion of the two reproductive modes varied according to the positions in the colony (Fig. 3). A lower proportion of sexually mature worms was found in the tips of growing branches in *S. dysteri*. It may be explained in terms of 1) allocation of colony resources to growth rather than gamete production, as in numerous eu-colonial organisms [4, 5, 17, 19], or 2) individual age. The peripherally positioned worms in a pseudo-colony seem to be younger than the centrally positioned ones because asexually produced buds extend their tubes in an outer direction over the stock worm.

In the aggregating polychaete *Pygospio elegans*, as food availability decreased according to their density-increment, sexual reproduction was induced [20, 21]. Similarly, food availability may regulate the commencement of sexual and asexual reproduction in *S. dysteri*. The position of worms in



FIG. 7. An aggregation of 11 sexually reproduced juveniles of *Salmacina dysteri* in a petri-dish. Scale bar represents 1 cm.

a colony seems to affect the availability of suspended food: centrally located worms receive less food, while peripheral worms receive an adequate food supply. This may result in more sexual reproductive ability in central colony positions than peripheral ones. Subsequently, larger pseudo-colonies seem to contain a higher proportion of worms located centrally (below 2 cm from the edge of a pseudo-colony) than smaller pseudo-colonies.

Inter-colonial variation of reproductive modes

Generally, the proportion of sexually mature worms increased with the pseudo-colony size in *S. dysteri*, but, in a rare case, a small pseudo-colony showed a high proportion of sexual reproduction (Fig. 5). The small pseudo-colony seems to have been freshly damaged, so the highest ability of sexual reproduction might have been retained. This possibility could be tested by breaking of large pseudo-colonies. The broken pseudo-colonies still showed a significant sexual reproductive capability 2 weeks after the artificial break, but within 1 to 2 months, they increased the proportion of asexual reproduction to a degree comparable to that of pseudo-colonies of the same size class (see the result of experimental halving and Table 1).

The age of a pseudo-colony may be a factor affecting the reproductive mode (see Figs. 5 and 6). However, we concluded that the size of a colony is the most important factor affecting the reproductive mode, based on the result of experimental halving, induction of asexual reproduction in solitarily reared worms, and inter-colonial variation of reproductive modes (Fig. 5).

Factors leading to pseudo-colony formation in Salmacina dysteri

Bosence [3] and ten Hove [8] discussed the ecology of serpulid polychaetes and listed various factors which cause their aggregation. These factors are, physical environmental factors, larval aggregation, brooding, asexual budding, response to light in the larval stage, larval retention, limited substrate availability, biotic factors such as space competition or predation, and possibly high primary productivity. In *S. dysteri*, some of these factors might also be working in the pseudo-colony construction process. We could not test the effect of environmental factors on the pseudo-colony formation. However, the following factors seem important in pseudo-colony formation; brooding, retention of larvae near the parent pseudo-colony, settlement of larvae on conspecific pseudo-colonies, and budding. Larger colonies seem to have the capacity to produce and retain more larvae in their near vicinity (Fig. 4), and thus can grow more rapidly than smaller colonies. Aggregation during larval settlement did not always occur (Table 2). However, it will contribute pseudo-colony formation if partially.

ACKNOWLEDGMENTS

We are grateful to Dr. T. Kikuchi, and Dr. T. Yamasu, for

providing facilities and suggestions during this work. We also thank Mr. S. Nakamura, for his help in collecting worm colonies, Dr. Harry A. ten Hove and Dr. J. H. Bailey-Brock for critical reading of our manuscript and anonymous reviewers for their comments and advice. This work was partly supported by the Grant in Aid for Scientific Research on Priority Area (#204) 'Dispersal Mechanisms', and Priority Area (#319), Project "Symbiotic Biosphere: An Ecological Complexity Promoting the Coexisting of Many Species" from the Ministry of Education, Science and Culture, Japan.

REFERENCES

- Berkeley E, Berkeley C (1954) Notes on the life history of the polychaete *Dodecaceria fewkesi* (nom. n.). J Fish Res Bd Canada 11: 326-334
- Boardman RS, Cheetham AH, Oliver, Jr WA (1973) Introducing coloniality. In "Animal colonies" Ed by Boardman RS, Cheetham AH, Oliver, Jr WA, Stroudsburg, PA Dowden, Hutchinson & Ross, pp v-ix
- Bosence DWJ (1979) The factors leading to aggregation and reef formation in *Serpula vermicularis* L. In "Biology and systematics of colonial organisms" Ed by Larwood G, Rosen BR, Academic Press, London, New York and San Francisco pp 299-318
- Brazeau DA, Lasker HR (1990) Sexual reproduction and external brooding by the Caribbean gorgonian *Briareum asbestinum*. Mar Biol 104: 465-474
- Connell JH (1973) Population ecology of reef-building corals. In "Biology and ecology of coral reefs, Vol 2, Ed by Jones OA, Edean R, Academic Press, New York, pp 205-246
- Faulkner GH (1930) The anatomy and the histology of bud formation in the serpulid *Filograna implexa* together with some cytological observations on the nuclei of the neoblasts. J Linn Soc London 37: 109-189
- Gibson PH (1977) Reproduction in the cirratulid polychaetes *Dodecaceria concharum* and *D. pulchra*. J Zool London 182: 89-102
- ten Hove HA (1979) Different causes of mass occurrence in serpulids. In "Biology and systematics of colonial organisms" Ed by Larwood G, Rosen BR, Academic Press, London, New York and San Francisco, pp 281-298
- Huxley TH (1855) On hermaphrodite and fissiparous species of tubicolous annelid *Protula dysteri*. Edinb Phil J New Ser 1: 113-129
- Knight-Jones EW, Moyses J (1961) Intraspecific competition in sedentary marine animals. Symp Soc Exp Biol 15: 72-95
- Larwood G, Rosen BR (1979) The biology and systematics of colonial organisms. Academic Press, New York and London, 589 pp
- Nakamura S (1984) Record of air temperature, surface water temperature and chlorinity at Sesoko in 1983. Galaxea 3: 105
- Nishi E (1993) Notes on reproductive biology of some serpulid polychaetes at Sesoko Island, Okinawa, with brief accounts of setal morphology of three species of *Salmacina* and *Filograna implexa*. Mar Foul 10: 11-16
- Nishi E, Nishihira M (1992) Colony formation via sexual and asexual reproduction in the serpulid *Salmacina dysteri* (Annelida, Polychaeta). Zool Sci 9: 1293 (abstract)
- Nishi E, Nishihira M (1993) Hermaphroditisms, brooding, and gamete production in the serpulid *Salmacina dysteri* (Polychaeta, Sedentaria). Publ Amakusa Marine Biol Lab 12: 1-11.
- Nishi E, Yamasu T (1992) Brooding and development of a serpulid tube worm *Salmacina dysteri* (Huxley) (Annelida, Polychaeta). Bull Coll Sci, Univ Ryukyus 54: 107-121
- Rinkevich B, Loya Y (1979) The reproduction of the Red Sea

- coral *Stylophora pistillata*. II. Synchronization in breeding and seasonality of planula shedding. Mar Ecol Prog Ser 1: 146-152
- 18 Schroeder PC, Hermans CO (1975) Annelida: Polychaeta. In "Reproduction in marine invertebrates Vol 3" Ed by Giese AC & Pearse JS, Academic Press, New York & London, pp 1-213
- 19 Wallace CC (1985) Reproduction, recruitment and fragmentation in nine sympatric species of the coral genus *Acropora*. Mar Ecol Prog Ser 88: 217-233
- 20 Wilson Jr WH (1983) The role of density dependence in a marine infaunal community. Ecology 64: 295-306
- 21 Wilson Jr WH (1985) Food limitation of asexual reproduction in a spionid polychaete. Int J Invertebrate Repr Dev 8: 61-65
- 22 Yamanishi R. (1978) Occurrence of *Salmacina dysteri* at Iwaya, Awajishima. Nature study 24:9-11 (in Japanese)